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Development of sustainable fly management tools in an era of global warming

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Abstract

House flies (*Musca domestica*) are global pests of animal agriculture, causing major annoyance, carrying pathogens among production facilities and humans and thus have profound impacts on animal comfort and productivity. Successful fly control requires an integrated pest management (IPM) approach that includes elements of manure management, mass trapping, biological control, and selective insecticide use. Insecticidal control of house flies has become increasingly difficult due to the rapidity with which resistance develops, even to new active ingredients. Global climate change poses additional challenges, as the efficacy of natural enemies is uncertain under the higher temperatures that are predicted to become more commonplace in the future.

The two major objectives of this research project were: 1) to develop a cost-effective autodissemination application method of Pyriproxifen (PPF), an insect growth regulator, for controlling house flies; 2) to study the effect of increasing temperatures on the interactions between house flies and their principal natural enemies.

First, we collected several wild house fly populations in both countries and established that most of them are susceptible to PPF, although one population in each country showed initial signs of PPF-resistance. An important finding is that the efficacy of PPF is substantially reduced when applied in cows' manure. We also found that PPF is compatible with several common species of parasitoids that attack the house fly, thus PPF can be used in IPM programs. Next, we tried to develop "baited stations" in which house flies will collect PPF on their bodies and then deliver and deposit it in their oviposition sites (= autodissemination). The concept showed potential in lab experiments and in outdoor cages trials, but under field conditions the station models we tested were not effective enough. We thus tested a somewhat different approach – to actively release a small proportion of PPF-treated flies. This approach showed positive results in laboratory experiments and awaits further field experiments.

On the second topic, we performed two experimental sets: 1) we collected house flies and their parasitoids from hot temperature and mild temperature areas in both countries and, by measuring some fitness parameters we tested whether the ones collected from hot areas are better adapted to

heat. The results showed very little differences between the populations, both of flies and parasitoids. 2) A “fast evolution” experiment, in which we reared house flies for 20 generations under increasing temperatures. Also here, we found no evidence for heat adaptation.

In summary, pyriproxyfen proved to be a highly effective insect growth regulator for house flies that is compatible with it’s natural enemies. Although our autodissemination stations yielded disappointing results, we documented the proportion of flies in a population that must be exposed to PPF to achieve effective fly control. Both the flies and their principal parasitoids show no evidence for local adaptation to high temperatures. This is an encouraging finding for biological control, as our hypothesis was that the fly would be adapting faster to high temperatures than the parasitoids.

Summary Sheet

Publication Summary

PubType	IS only	Joint	US only
Reviewed	0	1	0

Training Summary

Trainee Type	Last Name	First Name	Institution	Country
Ph.D. Student	Biale	Haim	University of Haifa-Oranim	Israel
M.Sc. Student	Betelman	Kfir	University of Haifa-Oranim	Israel
M.Sc. Student	Johnson	Dana	USDA-ARS	USA
M.Sc. Student	White	Roxie	USDA-ARS	USA

Contribution of collaboration

The American and Israeli teams actively collaborated by periodic skype calls, continuous emails and mutual visits. During October 2015 Mr. Haim Biale, a PhD student from the Israeli team, spent two weeks with the American team in Gainesville, FL, performing various experiments directly related to the project. In September 2016, Dr. Elad Chiel (the Israeli co-PI) visited Dr. Chris Geden (the American co-PI). In this visit we had thorough discussions on the results thus far and progress directions, and set up an experiment together. Dr. Geden visited the Israeli team in May 2017. In the visit we had mutual presentations and discussions of the project, as well as a professional visit to two dairy farms.

The collaboration was manifested in several aspects:

1. Harmonization of experimental methods in both sides.
2. Performing identical tasks in both sides, results in larger data sets, from which general conclusions can be drawn, while at the same time enabling adjustments according to local conditions.
3. Joint publications: one has already been published, the second one is currently being revised prior to resubmission, and a third one is being written too.

Final scientific report

BARD project number 4701-14R

Principal investigators:

- Dr. Elad Chiel, Department of Biology and Environment, The University of Haifa-Oranim, Israel.
- Dr. Chris Geden, USDA, ARS, Gainesville, Florida, USA.

<u>Reminder of the research objectives</u> <u>(copied from the research proposal)</u>

The overall goal of this proposal is to develop new tools and collect essential data for sustainable IPM programs of flies. Specific objectives are as follows:

1. Develop pyriproxyfen (PPF) as a management tool for fly IPM programs:
   a) Establish the compatibility of PPF with the principal fly parasitoids.
   b) Conduct outdoor cage studies to optimize “attract & deliver” stations for PPF autodissemination.
   c) Field tests of the most promising PPF autodissemination stations on dairy farms in the U.S. and Israel.
2. Climate change and fly management:
   a) Determine the effects of climate warming on fly-parasitoid interactions.
   b) Identify heat-tolerant parasitoid populations in the U.S. and Israel.
   c) Update the existing simulation model of house fly population dynamics and management to provide realistic outcomes of management decisions under high temperatures.

Achievements

Objective 1d. This objective was not included in the original proposal. We added it when we started working on the research, as we realized that the first step in this research should be **to determine the PPF-susceptibility levels of wild housefly populations**.

- Israel. We tested the susceptibility of five wild populations collected on dairy farms from different geographical areas. The tests were performed with house fly's rearing medium (wheat bran, calf feed, water). Two populations were found to be highly susceptible with no fly emergence, two other populations were slightly less susceptible, with 0.2-0.5% emergence rates at 60 mg/kg PPF; the fifth population (from Ma'ale Hahamisha) was a bit more resistant, with 4.3% emergence at 60 mg/kg PPF and 0.3% emergence at 600 mg/kg PPF. In a following experiment (performed on dairy cow manure), we found that the LC_{50} of the least susceptible population is three times as high as that of the most susceptible one. Interestingly, LC_{50} values in manure were substantially higher than expected based on the previous tests with larval rearing medium.
- USA. Four field strains (from CA, MN, FL and NE) were tested and compared to a lab insecticide-susceptible strain. Adult emergence from treated pupae was similar among the insecticide-susceptible strain and field strains from CA, MN and FL. Flies collected from Nebraska were significantly more tolerant than the others, and had an LC_{90} that was 5.4 times higher than the insecticide-susceptible flies.

  When flies from the insecticide-susceptible strain were tested in fly larval rearing medium, the LC_{90} value was similar to that observed previously in the comparisons with four wild strains. By contrast, the LC_{90} value in manure from a commercial dairy herd and manure from a beef cattle teaching herd were 15 and 66 times higher, respectively, than in the lab medium.

Overall, our results show that PPF is still effective in controlling house flies, although initial signs of resistance are evident in some locations, possibly due to history of PPF usage. This highlights the need for PPF resistance management. Another important finding is that PPF activity is compromised in cows' manure.

Objective 1a.

The main conclusion in this section is that PPF is compatible with principal housefly parasitoids at concentrations <600 mg/kg, and is suitable for use in integrated pest management. More details:

- Israel. We tested the effects of three PPF concentrations PPF on three major parasitoid species: *Muscidifurax raptor, Spalangia cameroni* and *Spalangia endius.* The emergence rates of *M. raptor* and *S. endius* were compromised in the highest concentration (600 mg/kg), whereas *S. cameroni* was already affected at 60 mg/kg. The longevity and fecundity of all 3 species did not differ between the treatments.
- USA. Four parasitoid species (*Muscidifurax raptor*, *M. zaraptor*, *Spalangia cameroni*, and *S. endius*) were tested with six PPF concentrations (0-6,000 mg/kg). Emergence rates of *M. raptor* and *M. zaraptor* were slightly affected at the high doses of 6,000 and 600 mg/kg PPF. *Spalangia cameroni* and *S. endius* were unaffected at any concentration.

The results of sections 1a and 1d were published:

Biale H, Geden CJ, Chiel E. 2017. Effects of pyriproxyfen on wild populations of the housefly, *Musca domestica*, and compatibility with its principal parasitoids. Pest Management Science, DOI: 10.1002/ps.4638.

Objective 1b. (this objective was performed only in the USA, as stated in the proposal)

The original designs for autodissemination devices were problematic under field conditions. Flies were reluctant to alight on modified Captivator traps with PPF-treated covers over the openings. The Ridmax trap was not sufficiently sturdy to allow modifications to the design. The Arbico metal trap had the advantages of sturdiness and bottom entry but placement of the PPF-treated material was difficult because of the design of the top of the trap. In 2016 we fabricated new traps using 5 gallon pails that were cut in a way to allow insertion of a screen inverted funnel cone that fed into a vertical exit tube treated with PPF. Using results from year 1, we used a bait that was a combination of molasses and the commercial Farnam attractant. To increase the odds of flies contacting the PPF we also suspended pipe cleaners dusted with PPF powder directly over the bait at the base of the cone.

Objective 1c.

The first field test was conducted at a dairy farm near Beatrice, Nebraska. The fly population in this area was moderate. In this site we used “Captivator” traps with PPF-treated mesh covers (Fig 1a). The second field test was conducted at two California dairy farms near San Jacinto in September 2016. Conditions were dry and hot and fly populations were low. The test was conducted in the same manner as described for the Nebraska test, except that a different PPF delivery method was used (Fig. 1b).

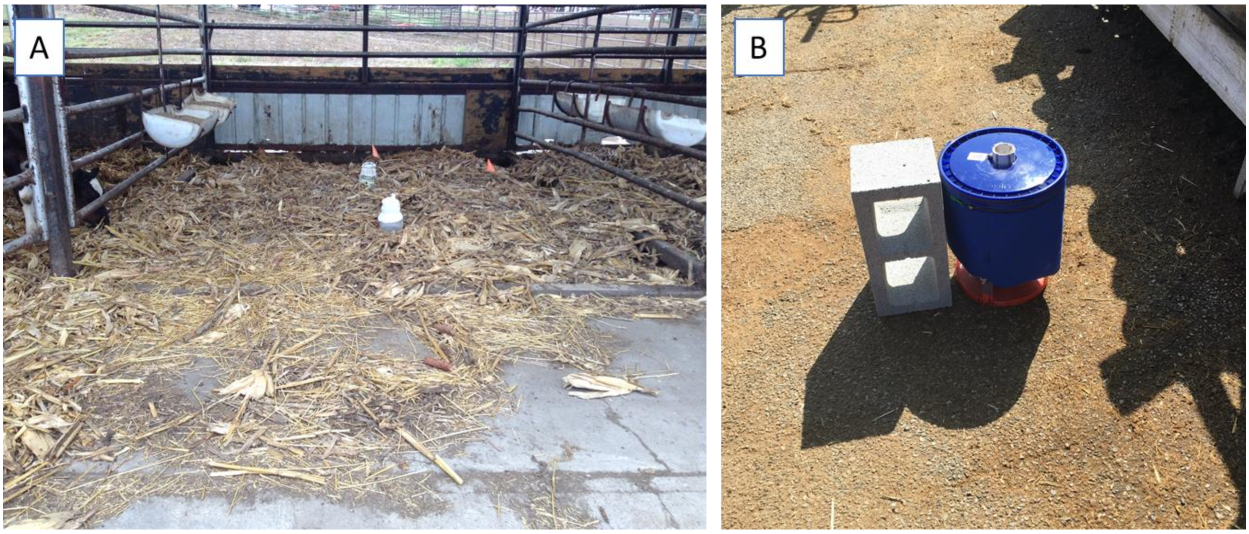

Figure 1. Designs of pyriproxyfen (PPF) autodissemination stations for house flies tested on dairy farms in Nebraska (A, left) and California (B, right). The Nebraska devices were Captivator fly traps with their respective bait, with the top of the trap covered with fabric dusted with a 48.5% PPF dust. California devices were composed of inverted cones placed over pans of fly bait with the top of cone removed to allow flies to pass through a cylinder lined with PPF-treated fabric. The cone was shrouded with dark fabric and covered with a plastic lid to encourage flies that entered the cone to move upwards toward the light and through the treated tube.

While conducting these assays we had many opportunities to observe the response of flies to the devices. In the large indoor cage study, using a long-established colony in an environment where the flies had no other objects to visit except the cage boundaries and the device, flies frequently approached and landed on the PPF-treated mesh on top of the baited Captivator traps. Wild flies on the Nebraska dairies, where there was a wide variety of food sources and resting sites, appeared reluctant to alight on the PPF-treated fabric. Flies were often observed to approach and fly around the devices but only a small number actually landed. The design of the device was revamped for the California tests in an attempt to compel flies to exit a cone through a PPF-treated exit tube. In practice we observed only a handful of flies that did so. Rather, flies were observed circling the baited pan and entering the cone, but generally either fell into the bait or exited the cone from below rather than using the PPF-lined exit tube at the top.

In light of these results and observations, we tested a new autodissemination approach: Instead of trying to lure flies to walk through PPF baits (which seems to be the bottleneck), we asked whether actively releasing certain proportions of PPF-coated flies would provide sufficient control. This approach would be technically- and economically-feasible if a low proportion of treated flies released will transfer sufficient amount of PPF to the oviposition sites (where also un-treated flies oviposit). We tested this concept in lab experiments with various manure types in the USA and in Israel. In the U.S study, 20% of PPF-coated flies were sufficient to get high control levels (~90%) in most of the tested manure types (dairy cows, egg-laying poultry, horse). In the experiments in Israel, fly mortality was low-medium in dairy cattle manure; however, in egg-laying poultry manure high pupal mortality was observed when only 10% of the flies were coated with PPF.

We conclude that autodissemination of PPF using the "active coating" concept may be practical, depending on manure type, location and fly population, and should be further tested in the field.

A manuscript that summarizes the results of sections 1b and 1c was submitted to Journal of Pest Science. It was recently withdrawn with recommendation to revise and resubmit. Currently we are working on the revision of this manuscript and will re-submit it soon.

Objective 2a.

- USA. This experiment was conducted in parallel with the Israeli team. Wild US flies were subjected to rearing under temperature regimes that ramped upward by 0.5^{o}C each generation. After 10 generations we saw no evidence for adaption to higher temperatures by the selected flies. By generation 15 the selected flies performed better than unselected flies under hot conditions but suffered from an overall cost in fitness. The final assessment, at generation 20, indicated that the flies were marginally more heat-tolerant than unselected flies, but fly survival at these hottest temperatures was extremely low. The final temperature regime was not as hot as is sometimes seen in the field, suggesting that field populations are able to mitigate the effects of high temperatures in ways that are difficult to duplicate in the lab.

- Israel. A multi-generational experiment, testing whether house flies adapt to elevating temperatures, was performed. For 17 generations house flies were reared either under moderate temperatures (daily fluctuations of 22-29°C, average 25°C) or high temperatures that elevate by 0.5°C in each generation (starting with 26-33°C daily, average 29°C). Fitness parameters were measured every 5th generation. No differences between the treatments were observed in generations 5 and 10. In the 15th generation (average temp 36.5°C) the fitness of the "heated" flies was significantly affected with no signs of adaptation (fecundity, longevity and emergence rates cut approximately by half). The next assessment was done on the 17th generation (because the "heated" flies were so poor, they probably wouldn't have survived to generation 20) and fitness parameters dropped further (e.g. fecundity was 1% of the control flies!). Bottom line- no adaptation to elevating temperatures has occurred throughout the experiment.

Objective 2b.

- USA. Collecting trips were made that resulted in the establishment of colonies of *M. raptor, M. zaraptor, S. cameroni* and *S. endius* from a historically cool location (Minnesota), a moderate temperature location (Nebraska), and a historically hot location near the Mojave Desert (California). Local Florida populations of all but *M. zaraptor* (which does not occur in Florida) have been colonized as well. A bioassay was developed to conduct high-throughput screening of these 15 colonies for heat tolerance from the standpoint of host attacks and oviposition. In this assay, groups of five parasitoids and >200 fly pupae are held together for 24 hours under either a constant temperature of 25ºC or two fluctuating regimes that simulate July temperatures in Coachella, California (high 41ºC, low 26ºC) or Ithaca New York (high 26ºC, low 14ºC). Two pupal presentation methods are tested for each combination. First, 200 pupae are presented without rearing medium. Second, parasitoids are introduced in 500 cc containers in which ca. 400 fly immature have developed and pupated. After 24 hours the parasitoids are removed and the pupae held at 27^{o}C for fly and parasitoid emergence. Three containers are tested for each parasitoid-temperature-pupal presentation combination per replication, and three replications (3 containers each) for each combination. The different species and strains varied considerably in their overall performance. *Muscidifurax zaraptor* and *S. endius* were substantially more heat tolerant than *M. raptor* and *S. cameroni*. There was no clear pattern of heat adaption in parasitoids from the hot California location. California *S. endius* had the highest heat tolerance for this species but ironically the Minnesota population of *M. zaraptor* was the most heat tolerant. The fly populations that were collected from the same four locations differed very little and showed no evidence for heat adaptation in flies from locations that are historically hot.

- Israel. Wild populations of house flies and parasitoids (*M. raptor*, *S. cameroni* and *S. endius*) were collected during the summer of 2017, from two geographical areas: the upper Galilee (moderate temperature location) and the northern Jordan valley (hot temperature location). Each population was divided and assigned to one of two climate-controlled incubators: 22.5°/29.5°C (moderate temperature regime) or 27°/38°C (hot temperature regime). The temperature regimes were chosen based on measurements we performed in the collection sites. In the incubators we measured the fecundity, sex ratio and longevity of each population. The null hypothesis was that the insects that were collected in the Jordan valley are better adapted to high temperatures. Results:
    - Flies. No significant differences were found between the populations in any of the measured parameters, under both temperature regimes. Flies from both populations lived longer under the moderate temperature conditions than under hot conditions, but this is not surprising.
    - Parasitoids. Generally, we found that fitness parameters were similar in parasitoids collected from both areas, with few exceptions: 1. under the moderate temperature regime, females of *S. cameroni* from the Jordan valley lived significantly longer than the ones from the Galilee; 2. geographical area had a significant effect on the longevity of *S. endius* and *M. raptor*: males from the Galilee lived longer than the ones from the Jordan valley under both temperature regimes; 3. Fecundity of *M.*

raptor from the Jordan valley was higher than the ones from the Galilee under both temperature regimes.

Overall, we found no sweeping evidence for a better heat-adaptation in the flies and parasitoids that were collected in the hot temperature location. This may be explained by high phenotypic plasticity.

A manuscript describing the results of 2a and 2b is currently in preparation.

Objective 2c.

Updating the simulation proved to be much more challenging than anticipated. The only extant versions of the original model from 25 years ago are in compiled C++ code and could only be run under Windows 1998 machines or earlier. More importantly, we were unable to locate any legacy versions of the original uncompiled source code. As a result, this subobjective would have required writing complete *de novo* code for all of the model's subroutines. This was beyond the technical expertise of the investigators.

Changes made to the original research plan

We added an additional set of experiments (labeled "Objective 1d"). The goal of these experiments is to determine the PPF-susceptibility levels of wild housefly populations.

Another aspect we added, on the Israeli side, is to reveal which microbial symbionts are harbored by common housefly parasitoids, in order to know how these may affect their effectiveness as biocontrol agents. The results were published (with a proper acknowledgement to BARD):

Betelman et al. 2017. Identification and characterization of bacterial symbionts in three species of filth fly parasitoids. FEMS Microbiology Ecology, 93.
doi: 10.1093/femsec/fix107

Publications for Project IS-4701-14 R

Status	Type	Authors	Title	Journal	Vol:pg Year	Coun
Published	Reviewed	*Biale Hain, Geden Chris, Chiel Elad*	Effects of pyriproxyfen on wild populations of the housefly, Musca domestica, and compatibility with its principal parasitoids	***Pest Management Science***	: 2017	Joint